REMARKS/ ARGUMENTS

Support for Amendments

Applicant amends claim 16 to correct a typographical error.

Response to Rejections

A. Claims 16-22, 24, 27, 28, 30-36 and 38 are not obvious over Nguyen et al. in view of Browne et al.

Claims 16-22, 24, 27, 28, 30-36 and 38 remain rejected under 35 U.S.C §103(a) as being obvious over Nguyen et al. (J. Biotech, 1999, 72, 115) in view of Browne et al. (Nature, 2002, 416, 38).

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 US 1, 148 USPQ 459 (1966) should be applied for establishing a background for determining obviousness under 35 U.S.C. §103(a), the factors including:

1) Determining the scope and contents of the references;
2) Ascertaining the differences between the references and the claims at issue;
3) Resolving the level of ordinary skill in the pertinent art; and
4) Considering the objective evidence present in the application indicating obviousness or nonobviousness.

Each factor is discussed in more detail for the convenience of the examiner.

Determining the scope and contents of the references

The first step is to determine the scope and contents of the cited references. The examiner states the Nguyen et al. reference (J. Biotech., 1999, 72, 115-25) teaches a

process for stabilizing immobilized antibodies by applying a solution of protein, 0.1% BSA, and a non-reducing disaccharide, 5% trehalose. The examiner further states the antibodies taught by Nyugen et al. are immobilized on polystyrene microtitration plates and are intended for use in an ELISA assay which is analytical and/or diagnostic. In addition, the examiner notes that Nguyen et al. do not teach the use of an LEA class protein in the stabilizing solution. In the obviousness rejection, the examiner omits the *specific requirement* of BSA and cites this reference as teaching the use of trehalose to stabilize biomolecules.

Referring to the Nguyen et al. experimental methods (also depicted in Fig. 1), Nguyen et al. formed a protector film by adding 0.1% BSA, 5% trehalose, 0.02% sodium azide and PBS to a microtitration plate and incubating the plate for 30 days at 4° C, room temperature, 37°C or 50° C. Referring to pg 119, col. 2,

> "The protecting property of the BSA-trehalose film appears to be a synergic action of BSA and trehalose because the BSA or trehalose alone will not maintain the immunoreactivity of these immobilized proteins (data not shown). The use of BSA as a protecting agent of protein during storage was established previously (Christensen and Riedel, 1980)."

Thus, it is the *relationship between BSA and trehalose* that was the finding in Nguyen et al. and not the use of trehalose to stabilize biomolecules. This relationship was further solidified by the observation that "the BSA or trehalose alone will not maintain the immunoreactivity..." *Thus, the scope and contents of Nguyen et al. provide that trehalose and BSA must be used in combination to stabilize biomolecules.* Thus any obviousness rejection incorporating Nguyen et al. must acknowledge the combination of trehalose and BSA and can not include trehalose and abandon BSA.

Now turning to Browne et al. (Nature 2002, 416, 38), the examiner argues Browne et al. teach that LEA proteins, together with trehalose and related sugars, stabilize biomolecules in plants during desiccation. Browne et al. is a brief communication published by Nature and is thus not a peer reviewed article. Referring to col. 1,

> "[Browne et al.] identified a gene in the anhydrobiotic nematode *Aphelenchus avenae* that is upregulated in response to desiccation stress and whose encoded protein shares sequence similarity with a late-embryonic gene that is induced in many plants when they are deprived of water. This finding suggests that animals and plants that undergo anhydrobiosis may use common protective strategies against dehydration, and provides a unifying insight into the mechanism of anhydrobiosis."

Thus, Browne et al. demonstrate there is a sequence similarity between a gene identified in *A. avenae* and LEA genes found in plants. In addition, there may be *in vivo* similarities between animals and plants to protect against dehydration.

The examiner also indicates that Browne et al. cite a reference by Wolker et al. (Biochimica et. Biophisica Acta 1544 (2001) 196-206) in support of the statement, "[s]ucrose glasses are stabilized *in vitro* by interaction with a purified group-3 LEA protein." Closer inspection reveals the sucrose glasses referred to in Wolkers et al. actually refer to experiments where an isolated pollen protein was dried in the presence of sucrose. These experiments were performed to evaluate the secondary structure of the pollen protein in the aqueous and dried state. Analysis of the dried pollen protein was performed using FTIR. *Thus, the Wolkers et al. reference did not demonstrate that sucrose and the pollen protein can stabilize a biomolecule immobilized on a surface in vitro, but instead showed the pollen protein's secondary structure can be affected by drying the pollen protein in the presence of sucrose. From these experiments, Wolkers et al. speculate sucrose and the pollen protein may function together within the cell cytoplasm to protect against effects associated with dehydration.* It should be noted that no experiment to assess whether protection or stabilization of a biomolecule actually occurs was performed. That is, there were no biomolecules tested in combination with the pollen protein and sucrose *in vivo* or *in vitro*. Instead, statements were based upon measuring the proportion of β-sheets present in the pollen protein when dried in the presence of sucrose. Therefore Wolkers et al. do not demonstrate the combination of a non-reducing sugar in combination with a LEA protein or polypeptide to stabilize a biomolecule.

Again, Wolkers et al. did not dry a biomolecule in the presence of sucrose and the pollen protein, but instead dried the pollen protein in the presence of sucrose. More specifically, Wolkers et al. isolated a protein from pollen, identified similarities to LEA proteins (including D-7 LEA protein from cotton) using BLAST searches, and performed FTIR analysis to further examine the secondary structure of the pollen protein in aqueous solution and in dry states. Using FTIR, Wolkers et al. found that the amount of β-sheet structures identifiable in the pollen protein were dependent on whether the sample was dried fast or slowly (*see* pg 202, col. 1). The pollen protein was also dissolved in a sucrose solution and evaluated in aqueous phase and dry states. Drying the pollen protein in the presence of sucrose resulted in fewer β-sheets and from this the authors suggest sucrose may prevent the formation of extended β-sheet structures found in the dried pollen protein (see pg. 202 col. 2). From these studies, Wolkers et al. speculate that the isolated pollen protein when in the presence of sucrose may provide stability <u>in the cytoplasm</u> of anyhydrobiotes during times of stress due to dehydration (see pg. 205, col. 1). *Thus, Wolkers et al. did not assess whether a biomolecule can be stabilized in vitro when in the presence of a pollen protein and sucrose, but instead Wolkers et al. speculate the pollen protein and sucrose may function together in vivo or in the cell cytoplasm to protect against dehydration effects within the cell.* Again, no study actually demonstrated the protection or stabilization of a biomolecule. For support, Wolkers et al. examined the secondary structure of the pollen protein dried in the presence of sucrose.

<u>Ascertaining the differences between the references and the claims at issue</u>

The second step is to ascertain the differences between the references and the claims at issue. In Nguyen et al., it was found that the synergic relationship between trehalose and BSA allows for the stabilization of immobilized antibodies. Thus, trehalose and BSA must be used in combination because as previously stated, "neither BSA or trehalose alone can maintain immunoreactivity of the examined antibody." Thus, there is no indication or suggestion for abandoning BSA.

Now referring to independent claim 16, from which claims 17-22 depend, the present invention does not use a combination of trehalose and BSA to maintain immunoreactivity of an antibody. More specifically, claim 16 includes a process for stabilizing or preserving a biomolecule, including the steps of providing a biomolecule immobilized on a surface and covering the surface with at least one non-reducing disaccharide and at least one protein or polypeptide of the LEA class. BSA is not required by claim 16. In fact, referring to pg 2, ll. 15-19 of the present application,

> "A further disadvantage of the above-described technology lies in the use of bovine serum albumin (BSA), a protein mixture of which it is known that, in the framework of antibody-aided applications, unspecific binding with antibodies results and thereby creates undesired cross reactions through which the entire experimental result is negatively influenced."

Thus Nguyen et al. require trehalose in the presence of BSA, whereas the present invention does not require trehalose in the presence of BSA. The present invention includes the use of a nonreducing disaccharide and a protein or polypeptide from the LEA class for the stabilization of an immobilized biomolecule. Furthermore the present application states that experimental results can be negatively influenced when BSA is used and thus technologies incorporating BSA are disadvantageous. Thus the significant differences between the Nguyen et al. technology and Applicant's technology must be reconciled in a proper obviousness rejection.

With respect to claims 24 and 27, from which 28, 30-36 and 38 depend, Applicant incorporates by reference the arguments set forth above in support of the position that the Nguyen et al. reference requires the use of *trehalose in combination with BSA*, whereas the present invention does not require BSA and in fact specifically points to the disadvantages of the use of BSA.

Now turning to Browne et al., which again was published without peer review, animals and plants may have similar protective strategies in response to dehydration conditions. The cellular response in plants was further examined by Wolkers et al., cited by Browne

et al. In Wolkers et al., experiments were performed to asses the folding characteristics of an isolated pollen protein in the presence or absence of sucrose. From examining the secondary structure of the dried pollen protein using FTIR, it was suggested that the pollen protein, when in the presence of sucrose, may assist in protection of cytoplasmic proteins *in vivo* when a plant is exposed to dehydration conditions. Wolkers et al. did not assess the ability of the pollen protein in combination with sucrose to determine the *in vitro* stability of a biomolecule and did not specifically test the stability of an *in vivo* or *in vitro* biomolecule.

In contrast, claim 16, from which claims 17-22 depend, includes a process for stabilizing or preserving a biomolecule, including the steps of providing a biomolecule immobilized on a surface and covering the surface with at least one non-reducing disaccharide and at least one protein or polypeptide of the LEA class. Thus, whereas Wolkers et al. specifically address cytoplasmic conditions that may occur during dehydration in plants, the present invention addresses *in vitro* applications of a non-reducing dissacharide used in combination with a LEA protein or polypeptide and their use for stabilizing or preserving a biomolecule immobilized on a surface.

With respect to claims 24 and 27, from which 28, 30-36 and 38 depend, Applicant incorporates by reference the arguments set forth above in support of the Wolkers et al. reference does not demonstrate the *in vitro* stabilization of a biomolecule immobilized on a surface including covering the surface or biomolecule with a composition including a non-reducing disaccharide and at least one protein or polypeptide of the LEA class. Wolkers et al. dried a pollen protein in the presence of sucrose to determine its folding characteristics. These significant differences must be reconciled in a proper obviousness rejection.

Resolving the level of ordinary skill in the pertinent art

The third step is to resolve the level of ordinary skill in the pertinent art. First, it should be noted one skilled in the present art would interpret the following Nguyen et al. passage as requiring BSA and trehalose in combination,

> "The protecting property of the BSA-trehalose film appears to be a synergic action of BSA and trehalose because the BSA or trehalose alone will not maintain the immunoreactivity of these immobilized proteins (data not shown)"

Secondly, it should be noted that those skilled in the present art tend to research authors in more detail when seeking additional information regarding an area of technology, its scope and corresponding limitations. Thus, while the examiner has indicated in the previous office action that a patent by Nguyen (US 5,264,831), which teaches away from a combination of the present invention, is not relevant because at least in part, the present invention does not specifically claim enzymes, the reference may be considered by one skilled in the art during routine investigation, to follow up or to understand the limitations of a technique. Thus, whether or not the present invention specifically claims enzymes, the Nguyen patent does identify limitations in a trehalose/BSA combination and should be considered when determining the state of the art or assessing one skilled in the art. Referring to Example 4 trehalose/BSA did not protect acetylcholinesterases of conger and torpedo fish as well as trehalose and gelatin (storage for 50 days at 50°C). Referring to col. 3, ll. 33-35, "[A]gents which are effective for protecting the immunologic activity of antibodies are not effective for enzymes." Thus, even with respect to the trehalose/BSA combination as set forth by Nguyen et al., it appears there are significant limitations to the Nguyen et al. technology that have not been observed with the present invention.

Considering the objective evidence present in the application indicating obviousness or nonobviousness

With respect to the Nguyen et al. paper, objective evidence in the present application clearly demonstrates a significant change in technical direction, which weighs heavily in favor of Applicant. Applicant herein incorporates by reference the description of the Nguyen et al. publication and the description of the present invention as provided above. In Nguyen et al., the authors expressly determine that trehalose and BSA should be used in combination to stabilize biomolecules. In the present application, Applicant specifically states that the use of BSA is disadvantageous. Thus with respect to Nguyen et al., Applicants have significantly changed the direction of technology incorporating immobilized biomolecules by providing a new composition for stabilization and preservation, one that includes a non-reducing sugar and at least one protein or polypeptide of the LEA class. This contradicts the Nguyen et al. reference and therefore weighs heavily in favor of Applicant's position.

With respect to Browne et al., which refers specifically to Wolkers et al., animals and plants may have similarities with respect to a protective response during dehydration. Applicant incorporates herein the description of the Browne et al. reference and the Wolkers et al. reference provided above. Neither Browne et al. or Wolkers et al. demonstrate the stabilization or preservation of an immobilized biomolecule using a non-reducing sugar and at least one protein or polypeptide of the LEA class. In Wolkers et al. a pollen protein was dried in the presence of sugar for FTIR analysis, which evaluated the secondary structure of the pollen protein. From this experimentation it was speculated that the pollen protein and sucrose may be used together in the cell cytoplasm to protect against effects encountered during dehydration. However, it should be noted that no actual stabilization or preservation of a biomolecule was actually demonstrated *in vivo* or *in vitro*. The absence of no actual study with respect to demonstrating a stabilized biomolecule weighs heavily in favor of Applicant's position.

Moreover as one skilled in the present art would recognize, the cell cytoplasm is a complex system. There are many pathways which result in the interaction of many proteins, membrane-associated molecules, hormones, RNA, cofactors, organelles, etc. Thus, even if sucrose and trehalose can act together in the cytoplasm, that does not necessarily mean they function alone and without other cytoplasmic molecules. The invention in the present application does not attempt to identify molecules involved in cellular regulation of pathways but instead the stabilization or preservation of a biomolecule immobilized on a surface. Thus even though Wolkers et al. dried a pollen protein in the presence of sucrose to identify its secondary structure, Wolkers et al. did not suggest or demonstrate the use of a non-reducing sugar and LEA polypeptide or protein for the stabilization or preservation of an immobilized biomolecule on a substrate or how such a process would be performed.

Thus in view of the above described differences between the cited references and Applicant's invention, Applicant respectfully requests the rejections be withdrawn.

B. If Nguyen et al and Browne et al were combined, the combination would yield results substantially different than the present invention

The examiner states that the present invention is obvious over Nguyen et al. in view of Browne et al.; however the full scope of the cited references must be determined for such a position. Just as the examiner notes that one cannot show nonobviousness merely by attacking the references individually where the rejections are based on combinations of references (see In re Keller, 642, F.2d 413, 208 USPQ 871 (CCPA 1981), "[o]ne can not use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to depreciate the claimed invention." In re Fritch, 972 F.2d 1260, 23 USPQ2d 1780, 1784. Thus, the references must read as a whole and the examiner can not selectively choose a phrase or word without acknowledging the full scope of its use.

For completeness, a logical combination of Nguyen et al. in view of Browne et al. is provided along with the required elements or results.

If in view of Nguyen et al, a first potential combination would require the use of both trehalose and BSA. It would not be proper to pick trehalose without BSA from the Nguyen reference. The relationship between trehalose and BSA is specifically described as synergic and experimental results by Nguyen et al. demonstrated they could not be used alone. Thus it would be improper to abandon BSA, and the proper scope of the Nguyen et al reference would include the use of both trehalose and BSA. In contrast, Applicants' invention does not require BSA when using trehalose.

If in view of Browne et al. or Wolkers et al., a second combination would require determining an *in vivo* application or merely drying out a protein in the absence and presence of sucrose for FTIR analysis of its secondary structure. In Wolkers et al., which was incorporated by Browne et al, sucrose and the pollen protein were speculated to protect against dehydration in the cell cytoplasm. Studies in support of this included drying out the pollen protein in the presence of sucrose and examining the pollen protein's secondary structure. Neither Wolkers et al. or Browne et al. demonstrate the stability of a biomolecule by adding a non-reducing disaccharide and a LEA protein, much less an immobilized biomolecule.

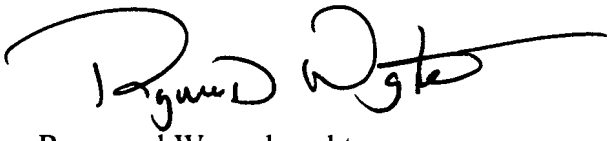
Thus, logical combinations of the Nguyen et al. and Browne et al. references would not yield the technological advances demonstrated in the present application.

C. International Preliminary Examination Report (IPER)

Lastly, Applicant points to the IPER for the parent PCT application, which indicated claims 1-9 and 11 would be considered inventive, those which include a composition for stabilizing or preserving biomolecules, comprising at least one non-reducing disaccharide and at least one protein or polypeptide of the LEA class. Although claims 1-9 have been canceled, pending claims 16-22, 24, 27, 28, 30-36 and 38 incorporate these elements and should also therefore be deemed inventive over the cited references.

Applicant respectfully requests all rejections be withdrawn and a notice of allowance issued for this application.

Respectfully submitted,



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